THE EFFECTS OF SPRUCE BIOCHAR ON SOIL FERTILITY AND BARLEY (*Hordeum vulgare* L.) YIELD FORMATION EIGHT YEARS AFTER APPLICATION TO NUTRIENT DEFICIENT SOIL

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Tiivistelmä Referat Abstract

Biochars are soil amendment materials produced via pyrolysis of biomass. They are resistant to degradation and can be used as a way to sequester carbon from the atmosphere. Biochars can improve soil structure and water and nutrient retention capacity, and significant positive effects on soil aggregate stability, water retention capacity and nutrient availability have been observed in acidic soils with low carbon content. The positive effects of biochar on soil properties can also increase crop yields. However, most studies on the effects of biochar have been conducted in tropical or temperate climates, and currently very little is known on its effects on the yield formation of cereals, and more specifically, barley.

The aim of this study was to determine the effects of softwood biochar on field soil moisture and nutrient contents, as well as its effects on yield components of barley (*Hordeum vulgare* L.) 8 years after its application (0, 5, 10, 20 and 30 t ha^{-1}) to boreal soil. In addition, the effects of organic and mineral fertilizers, alone and together with biochar, on soil moisture, nutrient contents and barley yield components were studied.

Biochar did not have significant effects on soil moisture or nutrient contents or on barley yield components. Fertilization had significant effects on contents of soil moisture and nutrients, electrical conductivity, pH and the biomass, leaf chlorophyll content, number and weight of seeds and the final yield of barley.

The non-significant effects of biochar can be due to the high amount of carbon already present in the soil, and similar results have been observed on the research site in previous years. The added biochar may also have been misplaced by soil management or degraded by weathering. The growing season of 2018 was drier and warmer than the long-term average and drought during the beginning of the growing season combined with issues with weeds negatively affected crop development and yield components.

Avainsanat Nyckelord Keywords
Hordeum vulgare L., barley, biochar, meat-bone-meal, mineral fertilizer, yield components

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Biohiiliä, eli pyrolyysimenetelmällä valmistettuja hiilipitoisia aineita, voidaan hyödyntää pitkäikäisenä maanparannusaineina. Biohiilien pitkä säilyvyys maassa mahdollistaa myös hiilen sitomisen ilmakehästä maaperään, mikä vähentää ilmakehässä olevan hiilidioksidin määrää. Biohiilet voivat parantaa maan mururakennetta ja vesi- ja ravinnetaloutta, ja erityisen suuria vaikutuksia niillä voi olla happamilla tai vain vähän hiiltä sisältävillä mailla. Biohiilien positiiviset vaikutukset maaperään voivat myös heijastua suurempiin viljelykasvien satoihin. Merkittävä osa biohiilien vaikutuksia käsittelevistä tutkimuksista on kuitenkin tehty trooppisessa tai lauhkeassa ilmastossa, eikä tietoa ole juurikaan saatavilla biohiilien vaikutuksista viljojen, ja erityisesti ohran satokomponenttien muodostumiseen.

Tämän tutkimuksen tarkoituksena oli selvittää kuusipohjaisen biohiilen vaikutuksia peltomaan vesi- ja ravinnetalouteen, sekä ohran (*Hordeum vulgare* L.) satokomponentteihin 8 vuotta biohiilen lisäyksen (5, 10, 20 ja 30 t ha^{-1}) jälkeen. Lisäksi tutkimuksessa selvitettiin eloperäisen ja mineraalilannoitteen vaikutuksia, yksin ja yhdessä biohiilen kanssa, maan ravinne- ja kosteuspitoisuuteen sekä ohran satokomponentteihin.

Biohiili ei vaikuttanut merkitsevästi maan kosteus- tai ravinnepitoisuuteen, eikä myöskään ohran satokomponentteihin. Lannoituskäsittelyt vaikuttivat merkitsevästi maan kosteus- ja ravinnepitoisuuksiin, johtolukuun, pH-arvoon, ohrakasvuston lehtivihreäpitoisuuteen, biomassaan, jyvien määrään ja painoon sekä satoon.

Biohiilen vähäiset vaikutukset voivat johtua maan korkeasta lähtökohtaisesta hiilipitoisuudesta, ja vastaavia tuloksia on myös saatu koekentältä edeltävinä vuosina. Lisätty biohiili on myös voinut kulkeutua syvemmälle maahan maanmuokkauksen yhteydessä, ja osa biohiilihiukkasista on voinut rapautua. Kasvukausi 2018 oli myös poikkeuksellisen kuiva, ja kevään kuivuus yhdistettynä suureen rikkakasvimäärään vaikeutti kasvuston kehittymistä, jolloin mitattava sato jäi tavanomaista pienemmäksi.

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Hordeum vulgare L., ohra, biohiili, lihaluujauho, mineraalilannoitus, satokomponentit

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ABBREVIATIONS AND CONCEPTS

B	Boron
C	Carbon
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cd	Cadmium
C/N ratio	Carbon-to-Nitrogen ratio
Cr	Chromium
Cu	Copper
DOC	Dissolved Organic Carbon
H/C ratio	Hydrogen-to-Carbon ratio
K	Potassium
LAI	Leaf Area Index
MBM	Meat Bone Meal
Mg	Magnesium
Mn	Manganese
N	Nitrogen
P	Phosphorus
PAH	Polycyclic Aromatic Hydrocarbon
Pb	Lead
S	Sulphur
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SON	Soil Organic Nitrogen
SPAD	Soil Plant Analysis Device
TDR	Time Domain Reflectometer

1 INTRODUCTION

Modern intensive agricultural production is facing challenges with the demand for increased yields to sustain the continuously growing human population. Increased production intensity can cause soil erosion and loss of water and nutrients from soil, and the use of agrochemicals contaminate soils and crops (IPCC 2014). Land degradation and expanding cities cause loss of arable soils and further pressure to increase farming in less suitable areas. In addition, increased CO_2 emissions from human activities can in the future increasingly cause challenges with changing global climate. Biochars have been shown to have many possibilities in improving or maintaining critical soil functions, semi-permanently sequestering carbon from the atmosphere and helping clean contaminated soils (Lehmann and Joseph 2015).

The positive effects of material similar to biochars have been known for over 2000 years, but only in recent decades has more research been done on its different effects on different crops. Biochars are products that are produced from organic matter at high temperatures. This process transforms the organic matter into a more stable form that resembles charcoal. Biochars can be produced from different feedstocks at different temperatures, which affect its final properties and effects on soil (Suliman et al. 2016).

Although biochars have in recent years been a topic of interest in sustainable agriculture, most studies focus only on short-term effects. However, long-term effects are needed to be studied to determine biochars' realistic potential in soil and crop yield improvement. Most biochar-related research has been conducted in tropical or temperate climates, and more information is needed on its effects in boreal conditions. In addition, not much is known on biochars' effects on cereal yield formation and yield components. Biochars mostly affect crops' yield components by influencing soil properties, such as increasing water retention capacity (Herath et al. 2013, Tammeorg et al. 2014a) and decreasing soil bulk density (Gighinji 2014, Glab et al. 2016), and by, for example, increasing the availability of nutrients by increasing microbial activity (Novak et al. 2009). Studies conducted in tropical or temperate climates suggest that biochars may in some cases increase grain yield in wheat (*Triticum aestivum* L.) (Solaiman et al. 2010), cob weight in maize (*Zea mays* L.) (Sara and Shah 2018) as well as increasing the number of tillers (Bakar et al. 2015) and panicles (Huang et al. 2019) in rice. (*Oryza sativa* L.) However, there is great variability in these effects, and in many cases, no observable effects on yield components of cereals have been observed (Tammeorg et al. 2014b, Reibe et al. 2015, Sänger et al. 2016, Hämäläinen 2018).

This study was done on the long-term effects of wood-based biochar on sandy boreal soils 8 years after its application. The targeted effects for this study were its effects on the yield formation and components of barley, an important crop in boreal conditions, on soil nutrient and water contents, as well as its combined effects with mineral and meat bone meal fertilizers. Currently there is very little research conducted on the effects of biochars' on yield components of barley, and almost no information is available on its effects in boreal farming conditions. This study was done in AgriChar research group at the University of Helsinki and was partly funded by Maj and Tor Nessling Foundation.

2 THE EFFECTS OF BIOCHAR ON YIELD COMPONENTS AND FORMATION OF BARLEY

2.1 Biochars

Biochar is term used for charcoal produced from various sources of biomass used in a way that does not allow rapid mineralization of the photosynthetically fixed carbon back to the atmosphere (EBC 2019). Biochars can be found both naturally in the soil and by human activities. Naturally occurring biochars are usually formed in forest fires, but they have knowingly been globally used as a soil enhancer by humans for over 2000 years. Traditional farming methods in many places have included, or still include, slash-and-burn cultivation for clearing forests and grasslands, and as well as improving soil properties (Lehmann and Joseph 2015). The oldest sites where human-made biochars can be found are *Terra Preta* soils in Southern-America, where over 2000-year-old biochars can still be found (Sohi et al. 2010).

The positive effects of biochars and their potential for use in sustainable agriculture have recently been of great interest, and the number of annually published research papers on biochars have increased from almost none to thousands in the past 20 years. Biochars can be used as soil amendments to increase agricultural productivity and to finding solutions to environmental challenges. Currently there are standardized products commercially available for agricultural use, and national and international collaboration between different stakeholders, such as researchers and production facilities, is steadily increasing. For example, the European Biochar Certificate (EBC) facilitates international product standardization of biochars and in Finland around 30 different Finnish municipalities and cities are using biochars in their pilot experiments in storm water management and landscaping. The Finnish Biochar Association has been active in summing together the activities to their website and Finnish Biochar map. The association also holds seminars and workshops, which have been attracting participants as far as from Egypt (SBY 2019). Although there are promising results from conducted research, more detailed information is still needed on its potential in different uses and in improving production of commercially available products.

2.1.1 Production of biochars

Biochars can be produced from any organic matter such as wood, crop residue, biodegradable waste or manure. Different feedstock and production temperature greatly affect the physical and chemical properties of the resulting biochars (Sohi et al. 2010, Lehmann and Joseph 2015, Suliman et al. 2016). Because of these differences in composition, standardization for production and quality of biochars has been made, for example, by European Biochar Certificate (EBC) and International Biochar Initiative (IBI). In certified biochar production, only material with carbon content >50 % are classified as biochars. All other material produced via pyrolysis, including the material produced from animal manure, are classified as pyrogenic carbon material (PCM) (EBC 2012).

Biochars are produced via pyrolysis. Feedstock is heated to 350-1000 °C in low oxygen or anaerobic conditions (EBC 2012) producing biochar, pyrolysis oil and different gases, such as carbon monoxide, carbon dioxide and methane. The gases produced from pyrolysis are considered potent greenhouse gases that should not be released into the atmosphere. Instead, these may be used as fuel (Sohi et al. 2010), which reduces the process' dependency on external energy sources.

The biochar produced from this process contains aromatic compounds, which affect its chemical properties, such as cation exchange capacity (Suliman et al. 2016) and electrical conductivity (Conz et al. 2017). The same type of feedstock pyrolyzed at different temperatures can in addition have different properties such as ash content or amount of volatile matter. In general, higher temperatures tend to reduce the number of volatile compounds and in turn increase the number of aromatic compounds (Wu et al. 2012, Zhang et al. 2015), which increase biochar stability in soil (Zhao et al. 2013, Kuzyakov et al. 2014, Conz et al. 2017). However, there is variability in the amount of volatile matter even when produced at the same temperature with the same type of feedstock. For example, the variation in these compounds in wood-based biochars can range between 28–61 %, but in manure-based biochars the variation is only 0–3 % (Enders et al. 2012).

In addition to pyrolysis temperature, feedstock properties affect the nutrient content and pH of biochars. In general, biochars produced from nutrient rich raw materials like animal manure contain more nutrients and ash than biochars produced from wood, and compost or crop wastes also have high variability in nutrient contents. Higher ash content increases the pH of biochar but reduce stability (Enders et al. 2012). In addition, biochars may also contain harmful compounds such as heavy metals or PAHs (Rombolá et al. 2019) depending on the type of feedstock and pyrolysis temperature. Particularly sewage-based biochars have been found to contain heavy metals (Song et al. 2014, Jin et al. 2016), and some, not EBC or IBI certified, biochars may produce long-term significant increases in soil PAH (Polycyclic Aromatic Hydrocarbon) levels after application (de Resende et al. 2018, Rombolá et al. 2019).

For agricultural use, it is of importance to know the different qualities of different biochars, as these greatly affect how the addition might influence soil properties. Properties of interest for agricultural producers can be particle size distribution, specific surface area, liming efficacy or pH, nutrient content, total C (Carbon) content and H/C (Hydrogen-to-Carbon ratio) ratio. More detailed properties that may be used in, for example, the reduction of the bioavailability of heavy metals or other toxic compounds, are surface area and cation exchange capacity (Lehmann and Joseph 2015).

2.1.2 Effects on soil properties

Biochars can influence the physical properties of soil, such as pore size, bulk density and aggregate stability depending on the structure of the target soil. For example, Burrell et al. (2016) found that, in a 3-year experiment (1 year in laboratory and 2 years of fallowing outside), biochars produced from woodchips, wheat straw (*Triticum aestivum* L.) and grape vine prunings (*Vitis vinifera* L.) at an addition rate of 3 % w/w all decreased bulk density and increased aggregate stability of coarse soil, but no similar significant effects were found in humus and nutrient rich soil (Chernozem). Soinne et al. (2014) found aggregate stability to increase with the addition of biochar produced from a mixture of Norway spruce (*Picea abies* (L.) H. Karst.) and Scots pine (*Pinus sylvestris* L.) (0, 15 and 30 t/ha) in clayey soils, but not for sandy soil. Significant decreases in soil bulk density of sandy soils were also found in other laboratory experiments (Githinji 2014, Glab et al. 2016) in addition to increased porosity (Githinji 2014) and soil water content (Glab et al. 2016, Haider et al. 2017). In field experiments, effects such as increased aggregate stability and porosity as well as increased volumetric water content have also been found (Cornelissen et al. 2013, Herath et al. 2013), though there is variance in the strength of different effects depending on target site (Ameloot et al. 2014, Fidel et al. 2019) and time after application (Tammeorg et al. 2014b), and these effects have also not always been apparent (Hardie et al. 2014).

Biochars’ effects on soil properties can also change over time. Changes in physical properties over time can be due to weathering and degradation of the added biochars (Naisse et al. 2014) or, for example, due to cultural practices such as plowing or harrowing. Disturbing the soil by plowing can move the particles deeper into the soil and dilute the concentration of the added biochars (Ameloot et al. 2014). This dilution or weathering of biochars have been proposed as some of the reasons why observed effects in field conditions may change or decrease over time.

The changes in soil physical properties also affect chemical and microbial processes in the soil, but they can also directly affect soil chemical properties by affecting soil pH, the availability of nutrients, contaminants and water, and by increasing nitrification rates via increased microbial activity (Novak et al. 2009, Jones et al. 2012). Biochars may also decrease soil C mineralization (Ameloot et al. 2014) and loss of soil C (Aller et al. 2018, Kätterer et al. 2019) due to their resistance to degradation. The different effects on soil chemical properties have been found to be due to biochars' large surface area, nutrient contents and cation exchange capacity (Lehmann and Joseph 2015) (Figure 1).

In addition to C, biochars contains nutrients, most importantly phosphorus (P) that influence the amount of nutrients in the soil. However, as biochars may also affect soil pH (Chan et al. 2007, Chintala et al. 2013, Wang et al. 2013, Liang et al. 2014) the amount of easily available nutrients to crops may either increase or decrease depending on the priming and/or liming effects of the added biochar. Nutrient cycling can in addition be enhanced by increased microbial activity (Jones et al. 2012, Sheng and Zhu 2018), reduced N leaching (Aller et al. 2018) and soil cation exchange capacity, though the rate of activity is also dependent on soil pH (Sheng et al. 2016) and, for example, cropping system (Fidel et al. 2019). Biochars have also shown potential to reduce bioavailability of harmful compounds, such as heavy metals or other toxic material in the soil (Lehmann and Joseph 2015). For organic contaminants, this is typically due to biochars' large surface area and porosity, which enables adsorption or sorption (Lehmann and Joseph 2015). For inorganic contaminants, the mechanism varies (Figure 1) depending on the type of compound, and in some cases, due to the added biochars' effects on soil pH or DOC.

In certain heavy metals, such as Cr (Chromium) or Pb (Lead), bioavailability may be reduced via coordination with functional groups ($-OH^-$ and -COOH) or by coprecipitation on mineral surfaces (Choppala et al. 2012, Lu et al. 2012). Bashir et al. (2018) found that biochar produced from maize crop residue, rice straw or rice husk all reduced the bioavailability of Cd (Cadium) in contaminated soil. Xu et al. (2017) also found that Cd, as well as Pb, bioavailability in soil was reduced by biochar. However, increasing soil pH or DOC (Dissolved Organic Carbon) may also in some cases increase contaminant mobility (Beesley et al. 2010).

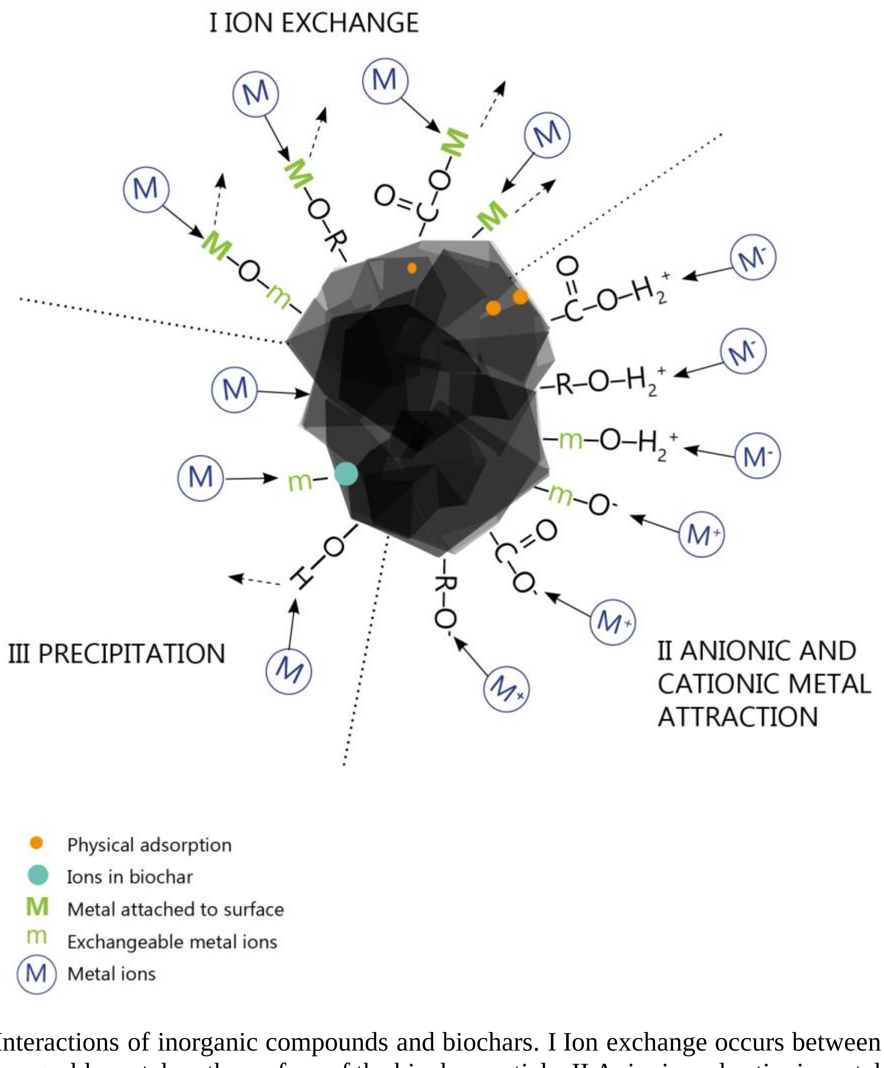

Figure 1. Interactions of inorganic compounds and biochars. I Ion exchange occurs between target metal and an exchangeable metal on the surface of the biochar particle. II Anionic and cationic metal electrostatic attractions on biochar's surface. III Precipitation of target metal. Physical adsorption occurs in pores. Adapted from Ahmad et al. (2013).

Biochars have also been proposed as a method for alleviating climate change via carbon sequestering. Plants actively sequester carbon from the atmosphere as a part of their photosynthetic process, but this is typically only a short-term carbon storage, as plants eventually die and decompose or are used as a source of energy by other organisms. This releases the carbon as CO_2 (Carbon dioxide) back into the atmosphere. In natural ecosystems, the release and uptake of carbon is mostly balanced, but current human activities have shifted the balance towards more carbon being released into the atmosphere than can be taken up by plants (IPCC 2014). For example, deforestation and the use of fossil fuels release previously sequestered carbon (IPCC 2014) and increasing climatic temperatures may release carbon into the atmosphere from permafrost soils due to increased microbiological activity (Bosch et al. 2017) or erosion (Raudina et al. 2018).

Biochars have shown potential for long-term carbon sequestering, as they are very resistant to degradation and is mostly composed of carbon. The efficiency of this depends on the net carbon released during the process by, for example, harvesting the material used, transportation and energy used for pyrolysis. In addition, soil properties may affect the realized efficacy of the sequestration, as low pH (Sheng et al. 2016, Sheng and Zhu 2018) or physical weathering (Naisse et al. 2014) may cause degradation of biochars, and thus increase CO_2 emissions from soil.

The actual effects in field conditions depend on the properties of the target site's soil, the quality and amount of biochars added and on other factors, such as farmland management and cropping history. For example, problematic soils with low (less than 10 g kg^{-1}) C-content have been shown to benefit more from biochars than soils that are not C-deficit (Sänger et al. 2016), likely because the added C in biochars act similarly than the native SOC (Soil Organic Carbon). In some field experiments with more fertile soils, statistically significant effects have only been obtained with higher (> 30 t ha^{-1}) biochar application rates (Liang et al. 2014, Prommer et al. 2014). In addition, in long-term experiments, the effects have often been found temporary or inconsistent, and results obtained in laboratories have not always been reproducible in field experiments. For example, Prommer et al. (2014) found that biochar decreased the cycling of field soil organic nitrogen (SON), but increased nitrification in the soil. Jones et al. (2012) found similar results in a greenhouse experiment, but these effects were not observed during a 3-year field experiment. Nelissen et al. (2015) also found inconsistent effects of biochar on soil properties in field conditions in a 2-year experiment, and similar inconsistent results have also been obtained by Cornelissen et al. (2013). In addition, in some cases biochars have also shown temporary negative effects on the availability of soil nutrients, such as N (Prommer et al. 2014). Biochars may, for example, temporarily reduce the amount of inorganic N in soil due to increased microbial activity that accelerate nitrification rates (Tammeorg et al. 2012; Prommer et al. 2014). In addition, biochar may adsorb chemical compounds (Figure 1), and for example Cu (Copper) may adsorb to biochar particles (Moore et al. 2018).

2.1.3 Effects on crop yield

The physical and chemical properties of biochars and their effects on soil can increase field crop yields particularly when added together with fertilizers to problematic soils. The main positive effects are increases in soil water retention capacity, nutrient availability, improved soil texture and changes in pH that in turn affects crop growth and development. In contaminated soils, biochars may also reduce the bioavailability of growth-reducing compounds (Xu et al. 2017, Bashir et al. 2018). However, in fertile soils or in soils with high contents of C, the effects of biochars on crop yield are often varying or negligible (Table 1).

Table 1. Some examples of field experiments on the effects of plant-based biochars or biochar + mineral or organic fertilizer on different crop yields in more fertile soils. The positive effects of biochars are often negligible or non-continuous. BC = biochar, MBM = Meat Bone Meal (Organic fertilizer).

Crop	**Soil type**	**Experiment**	**Treatments**	**Effects on yield**	**References**
Wheat, turnip rape and faba bean	Sandy clay loam	3 years, field	BC 0, 5 and 10 t/ha Mineral fertilizer 30, 65 and 100 % of recommended N level for each crop	No significant effects	Tammeorg et al. 2014a
Wheat	Loamy sand	2 years, field	BC 0, 5, 10, 20 and 30 t/ha Mineral fertilizer or MBM	No significant effects	Tammeorg et al. 2014b
Barley	Sandy loam	2 years, field	BC 0 or 20 t/ha	No consistent significant effects	Nelissen et al. 2015
Maize	Sandy soil	4 years, field	BC 0, 15 and 30 Mg/ha	No significant effects	Haider et al. 2017
Oat and barley	Silty clay loam	4 years, field	BC 8 and 25 t C/ha	No significant effects	O'Toole et al. 2018,
Maize/ Soybean	Alfisol	11 years, field	BC 22 Mg/ha	No consistent significant effects	Aller et al. 2018

In some cases, biochars have also been observed to affect crop nutrient contents. For example, Sänger et al. (2016) found that biochar application increased P, K and Mg content in wheat grains and Jones et al. (2012) observed an increase in foliar N of grasses. However, Alburquerque et al. (2013) found the addition of biochar to decrease N and Mn content in wheat when added alone to nutrient deficit soil, and Tammeorg et al. (2014) found N content of turnip rape (*Brassica rapa* subsp. *oleifera* L.) and wheat (*Triticum aestivum* L.) biomass to initially decrease with the addition of biochar to soil, but in many cases (Tammeorg et al. 2014b, Haider et al. 2017, Aller et al. 2018, Hood-Nowotny et al. 2018) no effects on the nutrient contents of crops have been observed.

2.2 Barley

Barley is one of the oldest cultivated food crops, being domesticated nearly 10 000 years ago in the Near East (Zohari and Hopf 2000). Modern-day production of barley is mostly concentrated on the production of malt or animal feed, though it is still economically one of the most important crops in Europe (Capettini et al. 2010).

2.2.1 Yield components and formation

The growth, development and yield formation of barley is similar to other common cereals (Slafer 2002), such as wheat (*Triticum aestivum* L.) and oat (*Avena sativa* L.). Yield components for cereals are the number of plants per hectare, number of spikes per plant, the number of seeds per ear and the weight of 1000 seeds (Guitard et al. 1961). Grain weight is significantly correlated with final yield, as the number of grains determines their weight (Slafer 2002).

Crop growth and yield formation can be divided into different growth stages. On the BBCH-scale (Meier 2001), crop development is numbered 0–99 (Figure 2). Numbers 0–9 indicate stages from seed germination to emergence. Numbers 10–19 indicate leaf development, where 10 is the emergence of the first leaf through the coleoptile. At 11 the first leaf is fully emerged, and leaf emergence continues until 19, where 9 or more leaves are present. Development stages from 20–29 are related to tillering, where 21 indicates that the first tiller is detectable, and increase in numbers the corresponding number of tillers emerged. Stages 41–49 are booting stages, where the formation of the flag occurs. Stages 51–69 are related to flowering. Inflorescences emerge between 51–59, and flowering occurs from 61–69. Fruit development and ripening of grains occur during development stages 71–89, where 89 indicated fully ripe grains. Crop senescing and harvest occur between stages 92–99 (Meier 2001).

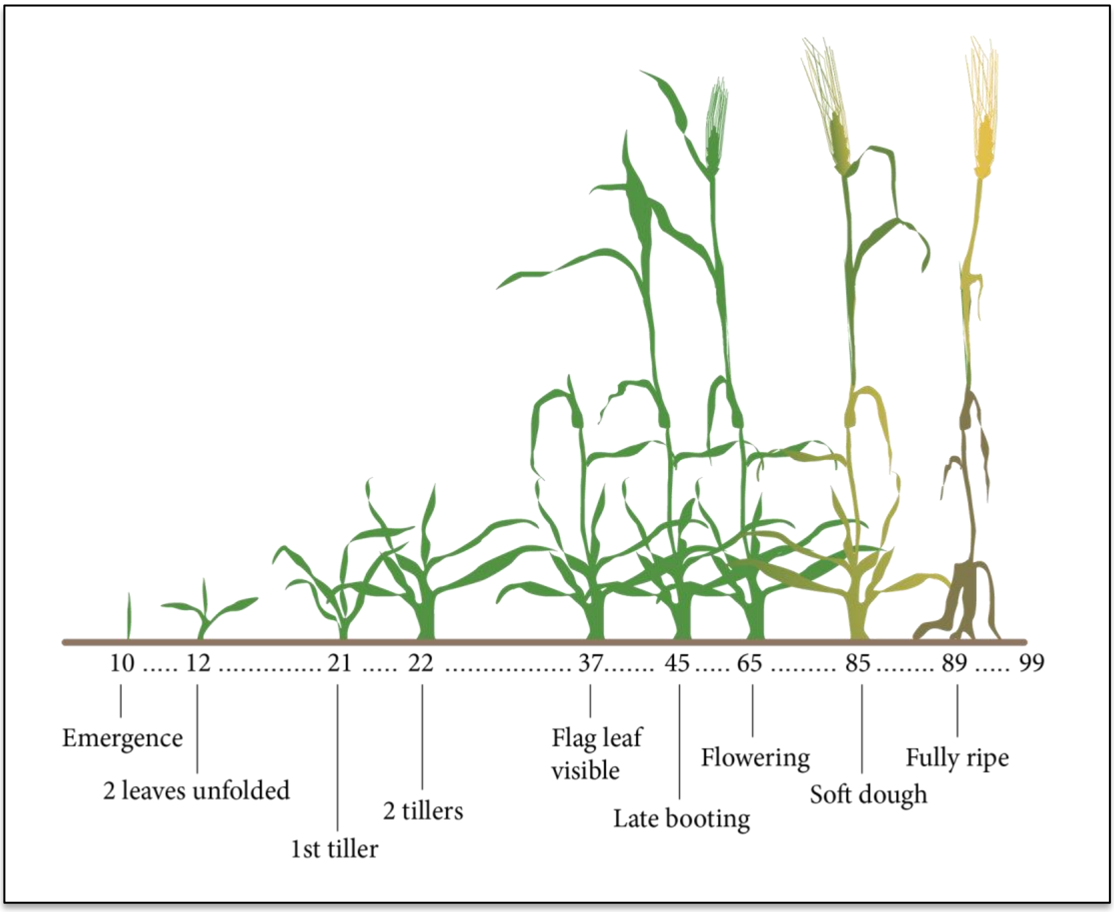

Figure 2. Certain stages of crop development according to the BBCH-scale. Adapted from Lancashire et al. (1991).

The final yield cannot usually be precisely estimated with the development of different yield components, as it is dependent on genetic, biotic and abiotic factors. Many modern varieties of crop plants have higher yield potential than older varieties (Capettini et al. 2010), but external and internal factors during different development stages also affect final yield. Abiotic factors are, for example, temperature and humidity, and biotic factors can be diseases or pest of the crop present in the ecosystem. In addition, nutrients play a key role as abiotic factors that greatly affect final yield.

In barley, final grain yield has been found to correlate with the number of tillers and above-ground biomass (Křen et al. 2014). A larger number of tillers and higher above-ground biomass increase leaf area index and thus photosynthetic potential, and photoassimilates can more readily be partitioned to requiring parts of the crop. Alaoui et al. (1991) found that even though barley often forms tillers that do not produce grains, these tillers may return up to twice as much dry matter to the main shoot as was provided to them.

Abiotic factors that negatively affect yield formation and components are terminal drought (Gonzáles et al. 1999, Samarah et al. 2009), drought during early growing stages (Hakala et al. 2012), high temperatures (Ponce et al. 1993, Hakala et al. 2012), waterlogging (Hakala et al. 2012) and nutrient deficiency.

Drought or nutrient deficiency prior to flowering can greatly decrease the number of grains per ear and grains per plant, (Aspinall et al. 1964, Slafer 2002, Samarah 2005) but grain size is more affected by drought during and slightly after flowering (Aspinall et al. 1964). Stem elongation is negatively affected by drought during booting stages and tillering temporarily or permanently decreases with stress caused by drought during these stages (Aspinall et al. 1964, Samarah 2005). High temperatures occurring during heading decreases yield, while low temperatures early in the growing season increase yield (Hakala et al. 2012). Lower temperatures at early development stages can lessen the negative effects of low precipitation, while higher temperatures during heading increase possible drought stress.

Studies done on wheat showed that waterlogging decreases the number of side tillers and seeds per ear (Watson et al. 1976, Collaku and Harrison 2002), as well as delays ear emergence and maturation in oat and barley (Watson et al. 1976) both in continuous waterlogging and temporary waterlogging during tillering or booting.

Nitrogen is one of the key nutrients needed for crop growth and development, and grain yield and N-contents are significantly affected by N availability (Oscarsson et al. 1998). However, N-uptake and effects on yield are also dependent on temperatures and rainfall during the growing season (Ponce et al. 1993). High temperatures combined with drought during grain filling decrease the effects of N fertilization (Ponce et al. 1993). In addition, high rates of added nutrients may also have negative effects on crop yield, and for example, a surplus of N at early growth stages may increase tillering but not the number of ears (Baethgen et al. 1995, Moreno et al. 2003), and high N availability after flowering can slow down grain filling and cause canopy lodging (Slafer 2002).

2.2.2 Barley and biochars

Biochars generally affect yield components indirectly by affecting conditions in the soil, especially when added together with fertilizers. For example, increasing soil water retention capacity via increasing aggregate stability (Herath et al. 2013, Soinne et al. 2014, Burrell et al. 2016) and decreasing soil bulk density (Gighinji 2014, Glab et al. 2016), decreases stress caused by drought. Barley is sensitive to drought during certain development stages, especially during tillering and prior to anthesis (Aspinall et al. 1964, Slafer 2002, Samarah 2005), and alleviating water related stress enhances yield formation. In addition, biochars may increase soil microbial activity (Novak et al. 2009, Jones et al. 2012) which can increase nitrification rates. This affects the availability of nutrients and crop growth, therefore potentially enhancing yield formation.

There have been studies conducted on the effects of biochars on barley yield with varying results, but very few studies focus the effects on specific yield components. In one study by Agegnehu et al. (2016) it was found that the number of tillers and leaf chlorophyll content significantly increased when 10 t/ha of acacia (*Acacia* spp. stem, branch and bark) biochar and compost (cattle manure and bedding, and crop residue) were added to Sub-Saharan Nitisol, and the effect was concluded to be due to improved soil water retention capacity, higher pH and increased SOC, contents of soil N, P, K, Ca and Mg (Nitrogen, Phosphorous, Potassium, Calsium and Manganese), as well as improved soil cation exchange capacity. However, as the yield components and formation of barley are similar to those of other cereals (Slafer 2002), possible effects of biochars on yield components of these could be similar in barley. In wheat, biochars have been observed to increase grain yield in some cases (Solaiman et al. 2010), but in other studies, no effects on yield components have been observed (Tammeorg et al. 2014b, Reibe et al. 2015, Sänger et al. 2016). In a field experiment by Sara and Shah (2018) biochar increased the cob weight of maize when added at a rate of 60 t/ha, and stover N content at a rate of 80 t/ha. Bakar et al. (2015) observed that soil amended with biochar (40 t/ha) from crop residues increased the number of tillers and yield in rice, and increased panicle size and number was also observed in rice by Huang et al. (2019) when added at a rate of 20 t/ha. Based on the results on other cereals, similar results could possibly be observed in barley too, depending on the target site.

More general studies on biochars' effects on barley yield also show that there could be potential effects to yield components, as final yield is dependent on them. For example, in tropical field conditions, Curaqueo et al. (2014) found that an addition of 20 t/ha of biochar from crop residue significantly increased barley grain weight in a 1-year study. Nowotny-Hood et al. (2018) found similar results in a 1-year experiment in temperate climate with biochar produced from hardwood at an application rate of 72 t/ha. However, O'Toole et al. (2018) did not observe significant effects of 8 t/ha grass-based biochar addition during a 4-year experiment in boreal conditions in Norway, nor did Nelissen et al. (2015) in a 2-year field experiment in Belgium in temperate climate conditions with wood-based biochar at an application rate of 20 t/ha.

Although there are studies done in tropical and temperate climates on the effects of biochars on the yield and yield components of different cereals research is still lacking on the effects of biochars on barley yield components. Boreal farming conditions differ from temperate and tropical climates, and currently very little is known on how different biochars affect yield formation and components of barley in these conditions.

3 RESEARCH AIM AND HYPOTHESES

The aim of this study was to determine long-term (eight year) effects of softwood biochar in loamy boreal soil, and possible combined effects of biochar and different fertilizers.

Research questions for this study were:
What effects do the different rates of softwood biochar, the type of fertilizer and the interaction of biochar and fertilizer treatments have on

(i) Content of moisture and nutrients in soil?
(ii) Yield formation of barley?

4 MATERIALS AND METHODS

4.1 Experimental design and study site

The study site, Vadelmakallio field, is located in Helsinki, Southern Finland (60° 13' 42'' N 25° 2' 34'' E) and is a part of teaching and research farm of University of Helsinki. Vadelmakallio field experiment site was established in 2011 (Tammeorg et al. 2014b). The experimental design is a factorial split-plot design containing four replicates (Figure 3 and Figure 4). Each main treatment plot is 6,6 m x 10 m, and subplot 2,2 m x 10 m.

Figure 3. Aerial image of Vadelmakallio field (Helsinki Karttapalvelu 2018) with replicates (I–IV) marked.

Plot number	Rep I		Rep II		Rep III		Rep IV
21		42		63		84	
20	**A3**	41	A2	62	D3	83	**C3**
19	**A1**	**40**	**A1**	61	D1	82	**C1**
18	A2	**39**	**A3**	60	D2	81	C2
17		38		59		80	
16	D3	**37**	**E1**	58	**A1**	79	D1
15	D2	36	E2	57	A2	78	D3
14	D1	**35**	**E3**	56	**A3**	77	D2
13		34		55		76	
12	B2	**33**	**C1**	54	B2	75	**A1**
11	B1	**32**	**C3**	53	B1	74	A2
10	B3	31	C2	52	B3	73	**A3**
9		30		51		72	
8	C2	29	B3	50	**E1**	71	E2
7	**C3**	28	B2	49	E2	70	**E3**
6	**C1**	27	B1	48	**E3**	69	**E1**
5		26		47		68	
4	**E1**	25	D2	46	C2	67	B3
3	E2	24	D3	**45**	**C3**	66	B2
2	E3	23	D1	**44**	**C1**	65	B1
1		22		43		64	

10 m corridor between replicates

Figure 4. Vadelmakallio field experimental design. The main plot factor is biochar application rate (A = 0 t/ha, B = 5 t/ha, C = 10 t/ha, D = 20 t/ha, E = 30 t/ha), and subplot factor fertilizer treatment (1 = control, 2 = MBM 100 kgN/ha, 3 = mineral fertilizer 100 kgN/ha). TDR-plots are marked with green colour and bolded text, empty plots (grey) are buffer plots only sown with barley.

Soil texture for the field has been classified as Endogleyic Umbrisol (WRB 2007) containing 83 % sand, 15 % silt and 2 % clay, with a wilting point of 8 % soil moisture content. The field has been prone to drought before the addition of the biochar in 2011 (Tammeorg et al. 2014b). After its establishment, the experimental field has been cropped with spring wheat in 2011 and 2012, a mixture of clover (*Trifolium pratense* L.) and timothy-grass (*Phleum pratense* L.) with barley as a cover crop in 2013, grass in 2014 and 2015, oat (*Avena sativa* L.) in 2016 (Hämäläinen 2018), and pea (*Pisum sativum* L.) in 2017.

Before the addition of the biochar in 2011, the soil had deficiencies in plant-available nutrients (Table 2). The pH of the soil and the content of P were classified as normal, but the soil was deficient of Ca, Mg, K and S. Soil electrical conductivity and pH were measured in an aqueous solution of 1:2,5, and nearly all C in soil was presumed to be organic due to only small amounts of carbonates present in soil (Tammeorg et al. 2014b).

Table 2. Soil nutrient analysis before the application of the softwood biochar in 2011 (Tammeorg et al. 2014b). Soil samples were taken at 0–20 cm depth from all plots.

Measurement	**Value**	
Conductivity	75.8	µS cm^{-1}
SOM	63.4	g/kg
pH	6.35	
P	20.6	g/m^3
Ca	1127.0	g/m^3
Mg	100.0	g/m^3
K	62.0	g/m^3
S	5.2	g/m^3
N_{total}	2.4	g/kg
$C_{organic\ total}$	31.7	g/kg
C/N ratio	13.2	
NH_4^+	6.2	g/m^3
NO_3^-	5.5	g/m^3
N_{min}	11.7	g/m^3

4.2 Biochar

The biochar used in this experiment was produced from Norway spruce (*Picea abies* (L.) H. Karst) at Preseco Oy in Lempäälä, Finland. Debarked spruce chips were pyrolyzed anaerobically at 550–600 °C for 10–15 min, and then let cool overnight in an airtight silo before being ground into < 10 mm particles (Tammeorg et al. 2014c).

Chemical and physical composition for the added biochar are presented in Table 3. According to Tammeorg et al. (2014c) nitrogen (N_2) adsorption (Micromeritics Co., Norcross, USA) was used to determine the surface area of the biochar. Dumas combustion method (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany) was used for C/N ratio analysis, and pH was measured in a 1:5 w/w suspension with deionized water. H/C ratio was determined by measuring the amount of hydrogen (CHN-1000, LECO, St. Joseph, MI, USA). Elemental analyses were conducted with an 1:10 v/v ammonium acetate extraction with a pH of 4.65 (Vuorinen and Mäkitie 1955). Dry combustion at 500 °C for 3 hours (Nabertherm Program Controller C19, Nabertherm, Lilienthal, Germany) was used for determining ash content, and volatile matter content by the mass lost during heating at approximately 900 °C for 7 min.

Table 3. Properties of biochar added to the experimental site in 2011(Tammeorg et al. 2014c).

Measurement	**Value**	
Surface area	265.0	m^2/g
pH	8.1	
C/N ratio	251.0	g/g
H/C ratio	0.3	mol/mol
Volatile matter	121.6	g/kg
$C_{organic}$	881.3	g/kg
C	882.5	g/kg
P	1.83	g/kg
Ca	4.66	g/kg
$CaCO_{3\ equivalence}$	9.0	g/kg
K	4.5	g/kg
Mg	0.9	g/kg
S	0.2	g/kg
N	3.5	g/kg
PAH	10.0	mg/kg
Ash content	26.6	g/kg

The biochar was applied to each treatment plot once with a sand spreader and mixed into the topsoil (0–10 cm depth) with a rotary harrow by two opposite passes (Tammeorg et al. 2014b). To reduce dusting, the biochar was moistened to 25 % w/w prior to application.

4.3. Growing season 2018

In 2018 the field and plots were tilled with rotary power harrow to 10 cm depth, and on 11th of May. The planned crop for the growing season was flax (*Linum usitatissimum* L. var "Abacus"), but due to drought it did not emerge and barley (*Hordeum vulgare* L.) was later sown instead. MBM (meat bone meal) or mineral fertilizers were added simultaneously with fungicide treated flax seeds with a seed drill according to the experimental design. Mineral fertilizer treatment received mixture of Yara Mila Hevi 6 N-P-K 14-3-15 (Yara International ASA) as the main fertilizer and 20 % of main fertilizer weight of Yara Starttiravinne M-P 12-23 (Yara International ASA) and 10 % Patenttikali (Lantmännen Agro Oy). The MBM fertilizer treatment consisted of Erikoisviljo N-P-K 8-4-8 (Honkajoki Oy, Honkajoki) as the main fertilizer and 10.5% of the main fertilizer weight added Patenttikali and 5.1 % of YaraBela Suomensalpietari (Yara International ASA). Such mixtures were used in order to provide equal amounts of N-P-K for both mineral and organic fertilizer treatments. In addition, 20 kg/ha of Mg (YaraVita MAGTRAC, Yara International ASA), 10 kg/ha of Mn (YaraVita MANTRAC PRO, Yara International ASA) and 0,5 kg/ha of B (YaraVita BORTRAC 150, Yara International ASA) were sprayed on all the plots resulting thus small amount of fertilization provided also to unfertilized plots (Table 4). The fertilizers were added to 5 cm depth and the seeds were sown at 3 cm depth with a fertilization seed drill. Due to the high amount of MBM fertilizer mixture needed, the fertilizer was applied in two passes with seeds sown only with the latter one. Thus, also the other experimental plots were passed twice to avoid possible effects of double compaction of the soil in MBM plots compared with other fertilizer plots. For MBM plots, fertilizers were added on both passes, but for mineral fertilization plots the seed drill did not apply any fertilizer on the first pass. After sowing, any visible seeds were covered with soil by manual harrowing. The added nutrients are listed in Table 4.

Table 4. Easily available nutrients from fertilizers (kg/ha) added to plots in 2018.

	N	P	K	S	Mg	B	Cu	Mn	Ca	Zn
Mineral	53	23	110	75	43	0.6	0.2	10	0	0
MBM	53	23	110	65	41	0.5	0.002	10	56	10
Control	3	-	-	-	20	0.5	-	10	0	0

Due to abnormal drought, the flax did not emerge. 3 l/ha of herbicide (Pilot Ultra, Nissan Chemical) was sprayed 7.6.2018 to reduce the amount of couch grass (*Elymus repens* L. Gould). Barley (var. "Harbinger") was sown 14.6. into the plots at 5 cm depth. Prior to sowing, all weeds were shredded, and plots were rotary power tilled at 3 cm depth to keep the fertilizer granules applied for flax in place. The barley emerged on the 20.6.

Pesticide treatments for aphids (*Aphididae* spp. Latreille) were done when needed. Noise strings were also added around the plots to scare off the geese that threatened to damage the crop. Further herbicide treatments were Primus 0,1 l/ha (Dow AgroSciences) with Sito Plus 0,1 l/ha (Berner Oy), and Express 75 DF 7 g/ha (Nordisk Alkali Biokemi A/S), but the experimental field remained weedy throughout the growing season (Figure 5).

Figure 5. Field experiment 10th of July 2018. The research field was very weedy despite both mechanical and chemical weed management.

The growing season of 2018 was drier and warmer than the long-term average (Figure 6). In May 2018 the average temperature was 4 °C higher than the long-term equivalent, and precipitation 28,20 mm lower. The weather conditions combined with the soil properties of the experimental field led to problems with crop emergence, as flax does not emerge and establish well in dry weather. Temperatures in June were closer to the long-term average, but temperatures in July, August and September were higher. Rainfall was unevenly distributed and less than average throughout the growing season until September. For example, in June, most of the precipitation occurred between 19.6. and 23.6. (Farmit 2018).

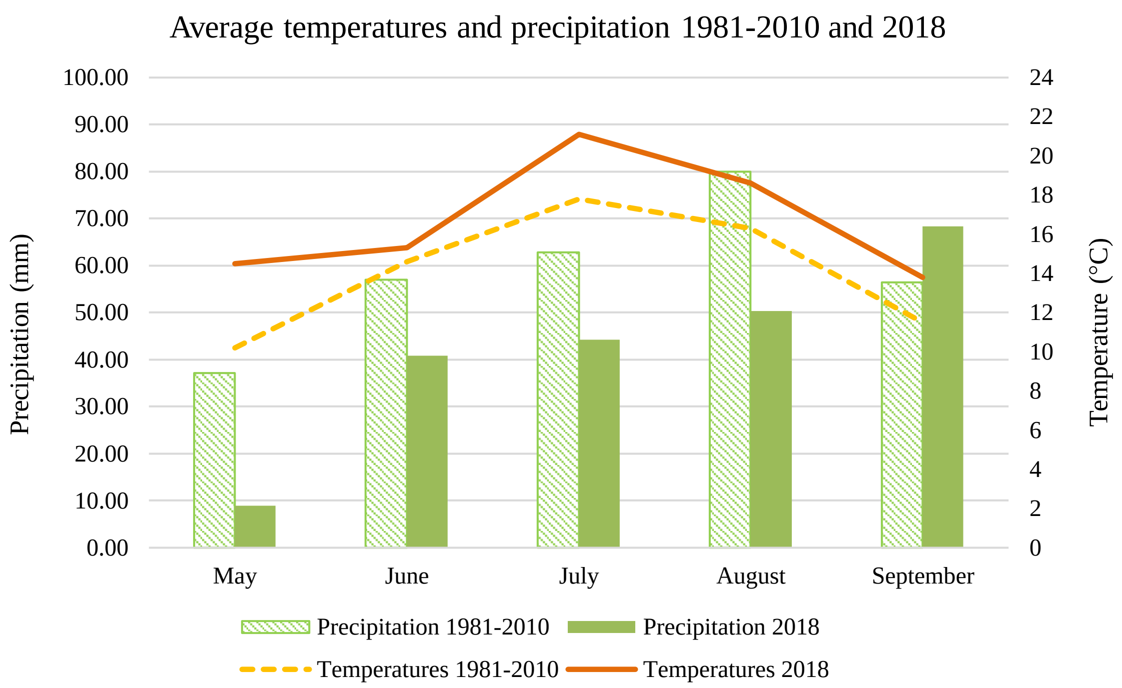

Figure 6. Average temperatures and precipitation in Helsinki, Kumpula 1981–2010 (Finnish Meteorological Institute 2018) and 2018 (Farmit 2018). May 2018 was notably dry with 28 mm less precipitation than the long-term average. Temperatures in growing season 2018 were on average 2 °C higher than the long-term average.

4.4 Measurements

4.4.1 Measurements and samples from field

The soil and crop properties were regularly monitored during the growing season (Table 5). The areas for stand count and samples, as well as SPAD measurements and soil samples, were chosen randomly, but representatively. No samples were taken less than 2 rows in from the sides of the plot, or less than 1 m in from the end of the plot. Areas without sufficient establishment, or otherwise non-representative, were also avoided.

Table 5. Measurements and samples from field in 2018

Type	Date(s)	Details	Measuring device
Cropping density	29.6.2018	3 samples from each plot. Number of stands, 30 cm of one row in each plot	
Development stages	27.6.2018–5.9.2018	Once per week	BBCH-scale (Meier 2001)
LAI	10.7.2018	4 measurements from each plot	SunScan SS1
Harvest	5.9.2018	Each plot harvested separately	Combine harvester
Plant samples	10.7.2018 10.8.2018 30.8.2018	3 samples from each plot, 30 cm of one row in each sample	
Soil sample	7.9.2018	16 samples from each plot at 0–20 cm depth	
SPAD	10.7.2018 7.8.2018	20 measurements from each plot	SPAD-502 DL
TDR	31.5.2018–26.9.2018	Once per week at 15 cm, 28 cm and 58 cm depth. Plots marked in Figure 1	MiniTrase 6050X3

The plant stand density was estimated 9 days after emergence, before tillering. 3 measurements were taken on the eastern side of each plot by counting the number of plants in one row at a 30 cm long distance. The number of plants per measurement were used to estimate the number of plants per plot (plants /m2). Growth stages were visually estimated once per week using the BBCH-scale (Meier 2001) for cereals. The development of the canopy was varying in different plots and parts of the field, which made assessing development stages challenging (Figure 7).

Figure 7. Unevenly developing plant stand. Photo taken on 7th of August 2018.

Plant samples were taken from each plot three times (samples I, II and III) during the growing season. Sample I was collected before stem elongation, sample II at flowering, and sample III at maturity. Samples I and II were taken without roots by cutting the plants 2 cm above ground. Any leaves below 2 cm were left uncut. Sample III was collected with roots in order to facilitate the yield component analyses. All samples were collected into pre-weighed paper bags and dried at 60 °C for 72 hours before weighing to determine dry matter weight.

Relative chlorophyll content value measurements (SPAD) were done twice during the growing season, the first time at development stage 21–29 and the second time at development stage 52. By the second measurement, prolonged drought and poor water retention capacity of the soil had caused some wilting of leaves. SPAD measurements were used to estimate the stand's nitrogen content, as well as chlorophyll content (Markwell et al. 1995). Measurements were done with a SPAD-502 device (Soil-Plant Development, Minolta Camera Co. Ltd., Osaka, Japan) on each plot by taking in total 20 measurements from equally developed leaves in different parts of the plot, and then calculating the average of the measurements.

LAI measurements were done once during the growing season, at development stage 55 (Meier 2001). Each plot was measured 4 times with a SunScan SS1-device (Delta-T Devices Ltd, Cambridge, United Kingdom) to get the average value for each plot.

Soil moisture content was measured weekly at 15 cm, 28 cm and 58 cm depth with TDR (Time Domain Reflectometer, MiniTrase 6050X3, Soilmoisture Equipment, Santa Barbara, USA). The measured plots are marked in Figure 1.

Soil samples were taken at 0–20 cm depth from each plot with an auger. In total 16 samples were taken from each plot (3x4 from corners and 1x4 from the center of the plot). Samples were collected into a clean container and the soil was mixed evenly. The container, auger and other tools used in the sampling were cleaned with paper between plots with treatments B1–B3, C1–C3 and D1–D3. For plots with treatment A1–A3 and E1–E3, the container and tools were also disinfected with 70 % (w/w) ethanol to avoid microbial cross-contamination.

4.4.2 Measurements from collected samples

Dried plant samples were used for analyses of C/N ratio from samples I and II, and yield components from sample III. Samples I and II were ground through a 1 mm sieve. The C/N ratio was analyzed from the ground samples with Dumas combustion method (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany).

Yield components separated from sample III were number of stems, main shoots, side tillers, grains per ear and number of ears per plant. Roots were removed from the stems by cutting 1 cm above the roots, and leaves were separated from the stems. Ears, stems and leaves were then weighed. The number of seeds per plant was calculated by first separating seeds from ears with a laboratory thresher (Hege 16, Hans-Ulrich HEGE GmbH & Co, Waldenburg, Germany). After threshing, any remaining impurities were removed with pressurized air. A seed counter (Pfeuffer GmbH, Kitzingen, Germany) was used for counting the total number of seeds for each sample. The number of grains per ear was calculated from the measured number of ears and seeds.

Chemical analyses from collected soil samples were pH and available nutrients according to common Finnish practices (Vuorinen and Mäkitie 1955; Viljavuuspalvelu 2008) and total C and N analyses by VarioMax (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany).

4.5 Statistical analysis

A linear mixed model was used for examining the effects of fertilizer treatments, biochar application rates and combined effects of biochar and fertilizers on barley yield components, using initial soil C value (measured in spring 2011) as the covariate. Replications were the random variable and the fixed factors were the application rate of biochar, fertilizer treatment and their interactions. Split-plot analysis of the variance model was done. The Shapiro-Wilk test and visual plot checking were used to assess if the data residuals were normally distributed, and if not, Box-Cox transformation (Box and Cox 1964) was used to normalize it. Bonferroni correction was used for *post hoc* pairwise comparison, and statistically significant interactions of biochar and fertilization were estimated from post hoc results based on 95 % confidence intervals. Statistical analyses were done with IBM SPSS Statistics (v. 24.0 IBM Corp., Armonk, NY, USA), with significance level at $p < 0.05$.

5 RESULTS

5.1. Content of soil moisture

Any addition of biochar did not have significant effects on soil moisture on any of the analyzed measurement dates or depths (Figures 8 and 9 and Tables 6 and 7), although on 13.9. at 0–28 cm measurement depth, a difference in effect (p 0.110) was observed between treatment 10 t/ha and treatment 30 t/ha (p 0.117), where treatment 30 t/ha had 2.41 % higher soil moisture than treatment 10 t/ha. Although non-significant, some observed effects of biochar were present on the first measurement date (27.6.), where soil moisture content was highest for the maximum biochar addition treatment at both measurement depths. At 0–15 cm, the 30 t/ha biochar application rate had 2.46 % higher soil moisture content than the lowest value, which was the application rate of 10 t/ha biochar. At 0–28 cm measurement depth the maximum biochar application rate had 3.58 % higher soil moisture content than the control or the application rate of 10 t/ha biochar.

During the driest weeks of the measurement period, both biochar treatments (10 t/ha and 30 t/ha) had soil moisture close or below wilting point (7.1% and 7.9 %) on 1.8. and only slightly above wilting point (8.8 % and 8.2 %) on 15.8., while the control had soil moisture contents slightly above wilting point (8.3 % on 1.8. and 9.4 % on 15.8.) on both measurement dates (Table 6). At 0–15 cm depth, the 10 t/ha biochar treatment had the lowest soil moisture content throughout the measurement period, excluding on 15.8. Similarly, the 10 t/ha application rate had the lowest soil moisture at 0–28 cm measurement depth, excluding between 18.7. and 1.8.2018.

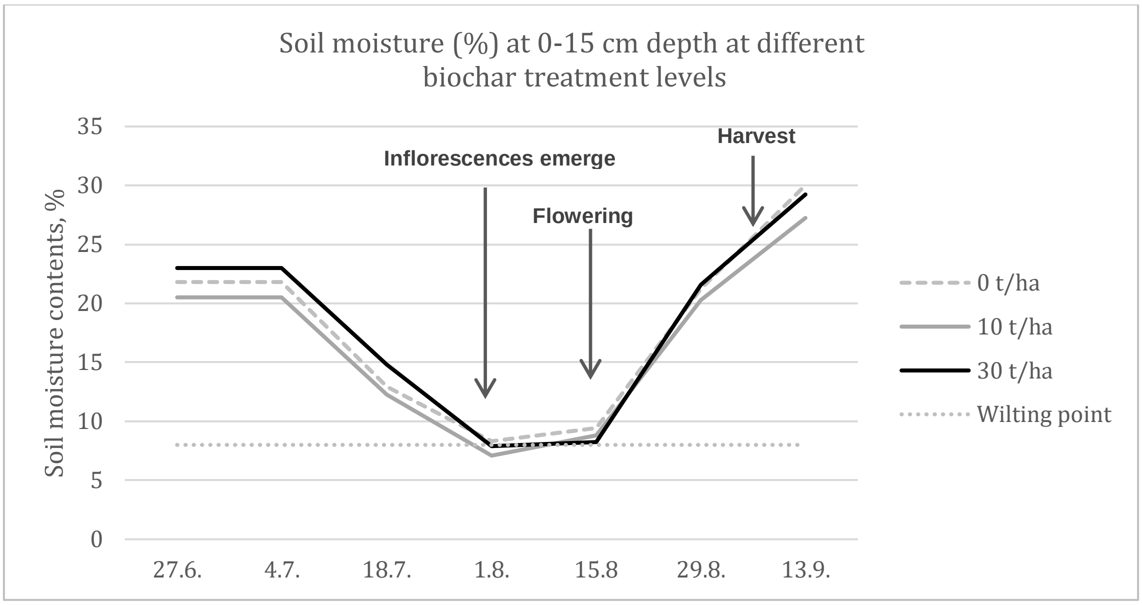

Figure 8. Measured content of soil moisture for biochar treatments 0, 10 and 30 t/ha at 0–15 cm depth on Vadelmakallio field in 2018. The results are the means from four replicates across three fertilization treatments (n = 12). Bonferroni correction was used for post-hoc pairwise comparison.

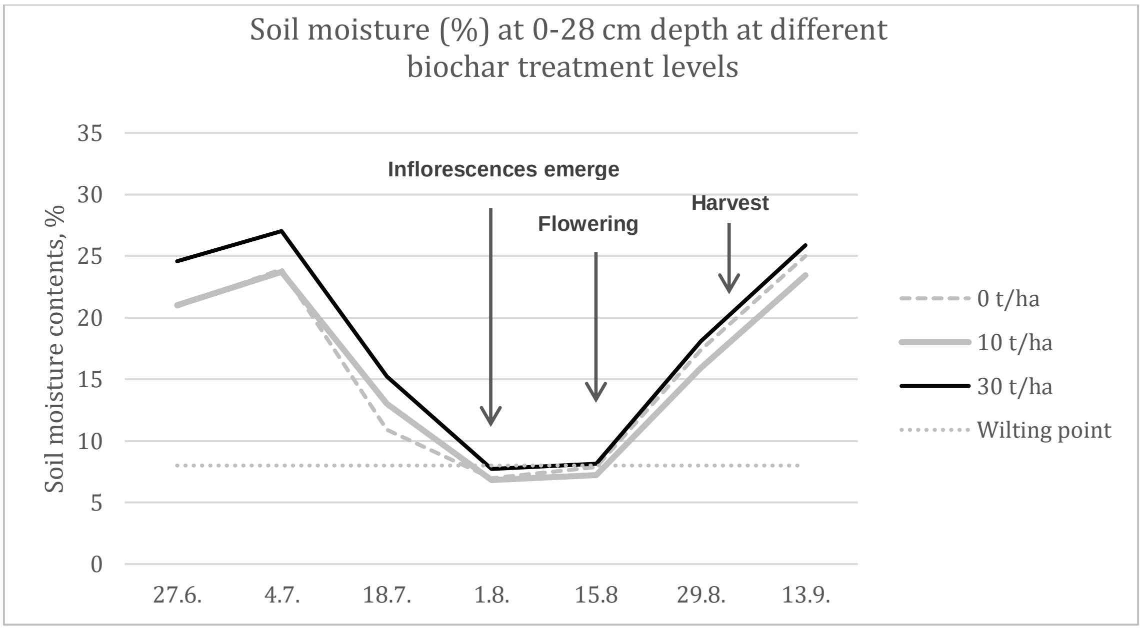

Figure 9. Measured content of soil moisture for biochar treatments 0, 10 and 30 t/ha at 0–28 cm depth on Vadelmakallio field in 2018. The results are the means from four replicates across three fertilization treatments (n = 12). Bonferroni correction was used for post-hoc pairwise comparison.

Fertilization increased soil moisture contents on measurement dates 27.6., 4.7. and 13.9. at soil depth 0–15 cm and on measurement dates 18.7. and 1.8. at 0–28 cm depth (Figures 10 and 11). A difference in effect was also observed on 15.8. (p 0.065) and 13.9. (p 0.058), where control plots had 0.70 % (15.8.) and 1.8 % (13.9.) higher soil moisture than plots with mineral fertilizers.

On 27.6. and 4.7., at depth 0–15 cm, soil moisture was 2.4 % (percentage points) higher for control plots than for plots with mineral fertilization, and 2.8 % higher for control plots than for mineral plots on 13.9. At measurement depth 0–28 cm, control plots had 2.1 % (18.7.) and 1.2 % (1.8.) higher soil moisture content than mineral fertilizer plots. On both measurement depths, control plots had higher moisture contents throughout the measurement period, and moisture below wilting point (8 %) was only observed on one measurement date (1.8.) at depth 0–28 cm. No interactions between fertilization and biochar were observed on any selected measurement dates or depths (Tables 6 and 7).

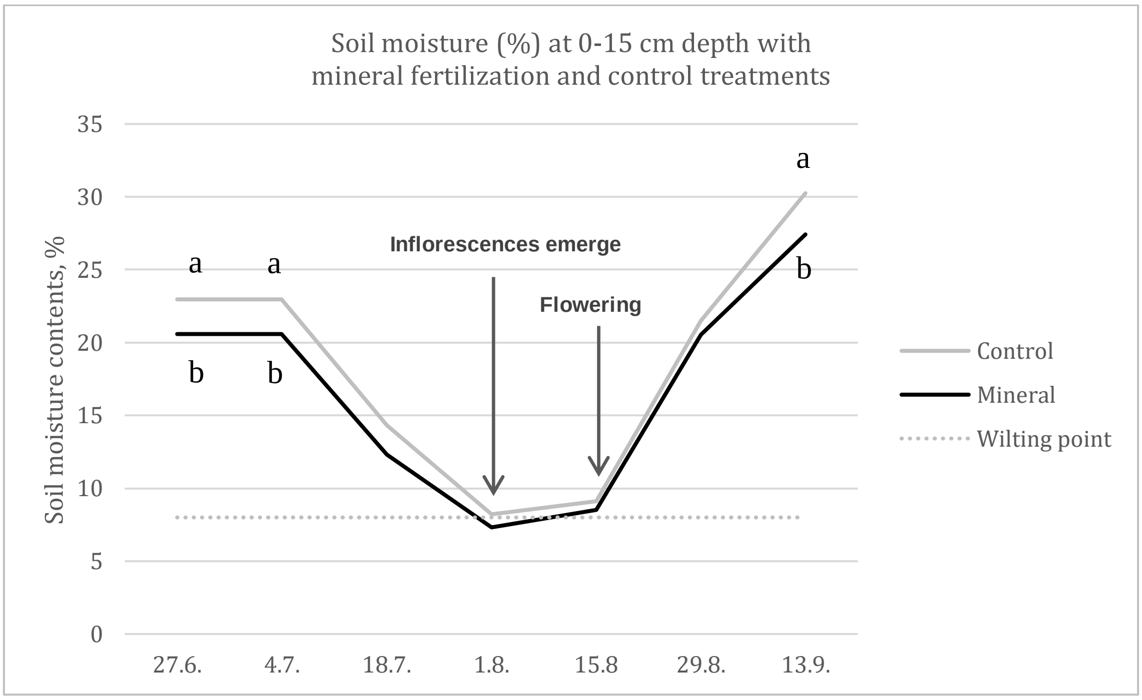

Figure 10. Measured content of soil moisture for mineral fertilization and control at 0–15 cm depth on Vadelmakallio field in 2018. The results are the means from four replicates across biochar levels (n = 8). Bonferroni correction was used for post-hoc pairwise comparison. Letters a and b indicate statistical significance between treatments.

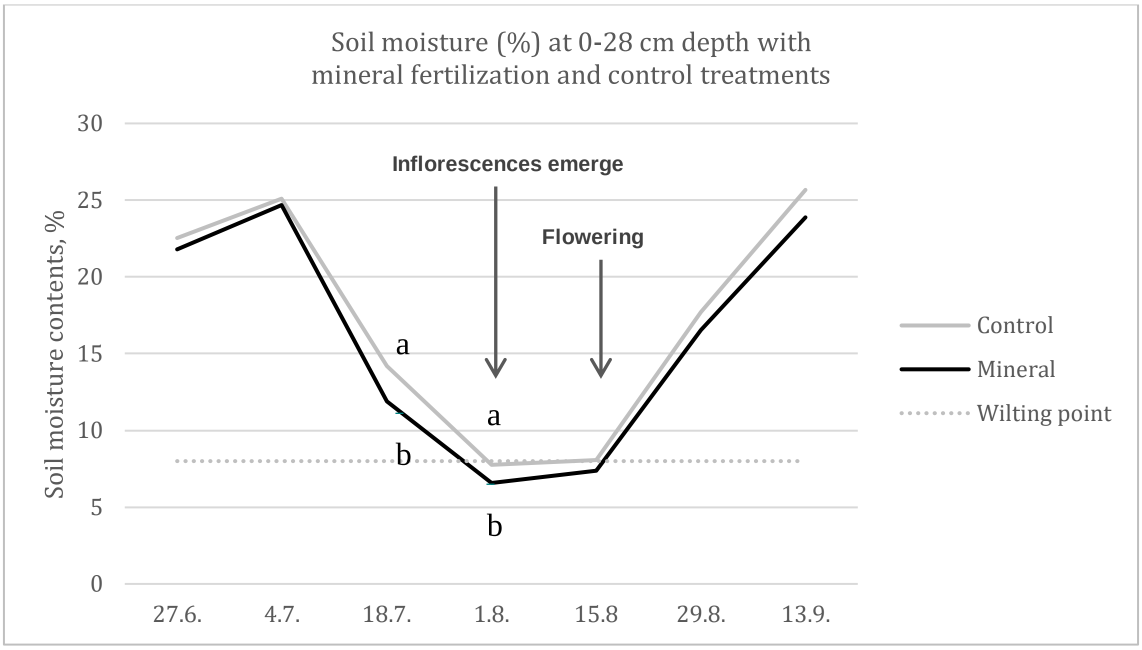

Figure 11. Measured content of soil moisture for mineral fertilization and control at 0–28 cm depth on Vadelmakallio field in 2018. The results are the means from four replicates across biochar levels (n = 8). Bonferroni correction was used for post-hoc pairwise comparison. Letters a and b indicate statistical significance between treatments.

Table 6. Soil moisture contents (%) at 15 cm depth. Treatments A–E indicate biochar (BC) application rates (A = 0 t/ha, C = 10 t/ha, E = 30 t/ha), and numbers 1–3 fertilization treatments (1 = control, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates across biochar treatments (n = 8). Letters after measurement values indicate statistical ($p < 0,05$) significance between treatments. Bonferroni correction was used for post-hoc pairwise comparison.

Treatment		**27.6.**	**4.7.**	**18.7.**	**1.8.**	**15.8**	**29.8.**	**13.9.**
A		21.84	21.84	12.92	8.32	9.44	21.24	30.02
C		20.52	20.52	12.23	7.09	8.78	20.28	27.25
E		22.98	22.98	14.81	7.89	8.23	21.57	29.24
1		22.97**a**	22.97**a**	14.34	8.22	9.12	21.51	30.25**a**
3		20.59**b**	20.59**b**	12.30	7.32	8.51	20.55	27.42**b**
	df	**p-values**						
BC	**2**	0.574	0.138	0.585	0.296	0.468	0.334	0.153
Fert	**1**	**0.002**	**0.007**	0.103	0.109	0.298	0.176	**0.012**
BC x Fert	**2**	0.152	0.812	0.933	0.527	0.571	0.803	0.981

Table 7. Soil moisture contents (%) at 28 cm depth. Treatments A–E indicate biochar (BC) application rates (A = 0 t/ha, C = 10 t/ha, E = 30 t/ha), and numbers 1–3 fertilization treatments (1 = control, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates across biochar levels (n = 8). Letters after measurement values indicate statistical (p < 0,05) significance between treatments. Bonferroni correction was used for post-hoc pairwise comparison.

Treatment		**27.6.**	**4.7.**	**18.7.**	**1.8.**	**15.8**	**29.8.**	**13.9.**[1]
A		20.89	23.92	10.91	6.96	7.85	17.37	25.00
C		20.99	23.71	13.01	6.81	7.24	15.94	23.45
E		24.57	27.02	15.20	7.71	8.12	18.08	25.86
1		22.52	25.09	14.20**a**	7.75**a**	8.09	17.73	25.66
3		21.78	24.67	11.89**b**	6.57**b**	7.38	16.53	23.88
	df	**p-values**						
BC	**2**	0.449	0.330	0.455	0.332	0.163	0.275	*0.110*
Fert	**1**	0.382	0.418	**0.047**	**0.019**	*0.065*	0.269	*0.058*
BC x Fert	**2**	0.882	0.981	0.981	0.323	0.657	0.271	0.548

[1]Data was not normally distributed even after 3 box-cox transformations, so original data was used

5.2. Content of soil nutrients

Biochar was observed to increase soil pH (p 0.043), and to have some observed effect on soil Ca contents (Table 8) (p 0.071, where biochar treatment 5 t/ha had lower Ca contents than the control with a significance of p 0.174) and to significantly increase soil C/N-ratio (p 0.040) (Table 9). However, for pH, Tukey HSD pairwise comparisons between biochar treatments, the lowest p-value was non-significant (p 0.092) (between control and 5 t/ha biochar, where the pH of the biochar treatment was lower). For soil C/N-ratio, pairwise comparison between biochar treatment types showed a significance of p 0.066 between treatments 10 t/ha and 30 t/ha, and a significance of p 0.168 between treatments 10 t/ha and 20 t/ha. Soil C/N-ratio was lower for treatment 10 t/ha than for treatment 30 t/ha (p 0.066), but not significantly lower for treatment 10 t/ha in comparison to treatment 20 t/ha (p 0.163).

Adding fertilizers significantly (p < 0.001) increased the electrical conductivity, pH and nutrient contents of Ca, P, K, S, B and Mn in the soil (Table 8). For Mg, the significance of fertilization was slightly lower (p 0.002) and no effects were observed for Zn or Cu (p 0.074).

Mineral fertilization increased soil conductivity more than MBM, but both fertilizer types increased soil conductivity in comparison to the control. Both fertilizer treatments slightly decreased soil pH in comparison to the control (pH of control was 5.97, while the mean pH of MBM was 5.86 and for mineral 5.81), but the differences between treatments were non-significant (p 0.061). For soil Ca contents, the difference between MBM and control was non-significant, but mineral treatment plots had less Ca than control and MBM plots. Soil P was significantly ($p < 0.001$) increased by MBM, and non-significantly increased by mineral fertilization. The difference in effect of treatments was significant (p 0.010), where MBM increased soil P more than mineral. Soil K, S and Mn were significantly ($p < 0.05$) increased by both MBM and mineral fertilizers, but mineral fertilizers increased soil K, S and Mn significantly in comparison to MBM. Only mineral fertilization increased soil B significantly, while MBM only slightly increased soil B in comparison to control, and the difference in effects of treatments for B were also statistically significant. For Mg, significant differences in effects were observed between control and both fertilization treatments, where both mineral and MBM increased soil Mg content.

Fertilization did not have significant effects on the content of soil C or N or on C/N-ratio (Table 9). Interactions between biochar and fertilization was observed for B-, Cu- and Mn-contents with p-values of 0.005, 0.050 and 0.024, respectively (Table 8). The highest biochar treatment combined with mineral fertilization increased soil B significantly, while biochar treatment 10 t/ha with no fertilization significantly lowered soil B contents. For soil Cu contents, biochar treatment 20 t/ha combined with mineral fertilization significantly increased soil Cu, while biochar treatment 10 t/ha with no fertilization significantly decreased soil Cu. For Zn, no significant interactions were present based on the comparison of interactions' upper and lower bounds (95 % confidence interval).

Table 8. Soil nutrient analysis. Treatments A–E indicate biochar application rates (A = 0 t/ha, B = 5 t/ha, C = 10 t/ha, D = 20 t/ha, E = 30 t/ha), and numbers 1–3 fertilization treatments (1 = control, 2 = MBM 100 kgN/ha, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates (n = 4). Letters after measurement values indicate statistical ($p < 0.05$) significance between treatments. Bonferroni correction was used for post-hoc pairwise comparison.

Treatment	**Electrical Conductivity ($\mu S\ cm^{-1}$)**	**pH**	**Ca (g/m^3)**	**P[1] (g/m^3)**	**K (g/m^3)**	**Mg (g/m^3)**	**S (g/m^3)**	**B (g/m^3)**	**Cu[1.1] (g/m^3)**	**Mn (g/m^3)**	**Zn (g/m^3)**
A	0.98	5.99	1132	21.53	77.47	114.91	16.62	0.47	11.53	11.75	18.80
B	0.82	5.78	949	19.47	79.56	96.70	15.37	0.46	13.24	14.20	21.68
C	0.91	5.83	968	23.26	81.28	103.73	15.31	0.46	10.78	12.34	18.77
D	0.86	5.97	1099	21.31	75.88	113.38	14.05	0.47	13.85	9.76	17.70
E	0.89	5.85	994	21.70	93.82	101.53	15.87	0.52	12.32	14.69	21.45
1	0.60**a**	5.97**a**	1050	18.55**a**	61.61**a**	102.33**b**	5.83**a**	0.45	11.76	10.80**c**	19.70
2	0.92**a**	5.87**a**	1047**b**	25.20**c**	86.39**a**	107.71	17.58**a**	0.47**b**	11.80	12.53**b**	19.59
3	1.15a	5.81	988a	20.61**b**	96.80**a**	108.11**a**	22.92**a**	0.50**a**	13.47	14.32**a**	19.75

	df	**p-values**										
BC	**4**	0.361	**0.043**	*0.071*	0.808	0.322	0.173	0.753	0.381	0.660	0.295	0.459
Fert	**2**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	*0.074*	**<0.001**	0.884
BC x Fert	**8**	0.651	0.374	0.815	0.745	0.282	*0.096*	0.955	**0.005**	**0.050**	0.391	**0.024**

[1]Data contained an abnormally high value of 65 g P/m^3 (plot 81) when values no higher than 33 g P/m^3 were observed on any other plot. The plot was removed from the data.

[1.1.]Data was not normally distributed even after 3 Box-cox transformations. so p-values from non-transformed data were used for these variables.

Table 9. Soil C, N and C/N-ratio analysis. Treatments A–E indicate biochar application rates (A = 0 t/ha, B = 5 t/ha, C = 10 t/ha, D = 20 t/ha, E = 30 t/ha), and numbers 1–3 fertilization treatments (1 = control, 2 = MBM 100 kgN/ha, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates (n = 4). No significant (p < 0,05) effects between treatments according to Tukey's post-hoc pairwise comparison.

Treatment		C (g/kg)	N (g/kg)	C/N
A		2.87	0.25	11.34
B		2.87	0.26	11.12
C		2.54	0.24	10.63
D		3.06	0.26	11.88
E		3.16	0.26	12.10
1		2.91	0.25	11.45
2		2.90	0.25	11.35
3		2.89	0.25	11.45
	df		**p-values**	
BC	**4**	0.163	0.432	**0.040**
Fert	**2**	0.975	0.749	0.554
BC x Fert	**8**	0.763	0.785	0.633

5.3 Barley leaf area index, C/N-ratio and SPAD value

In all treatments, the value for SPAD was lower on the later measurement date (Table 10). Significant ($p < 0.05$) effects of fertilizer treatments on SPAD were only observed in the first measurement for mineral fertilizers, where mineral fertilizer increased SPAD in comparison to MBM and control. MBM did not affect SPAD in comparison to the control (Table 8). Fertilization did not have any significant effects on the second SPAD measurement or on C- or N-contents, C/N-ratio or leaf area index. Biochar did not have any significant effects on any SPAD measurements or on C- or N-contents, C/N-ratio or leaf area index (Table 10). It also did not have any significant combined effects with fertilizers.

Table 10. Leaf SPAD values, C- and N-contents, C/N-ratio and leaf area index (LAI) means and p-values for different treatments. Biochar (BC) application rates are A–E (A = 0 t/ha, B = 5 t/ha, C = 10 t/ha, D = 20 t/ha, E = 30 t/ha) and fertilizer (Fert) treatments 1–3 (1 = control, 2 = MBM 100 kgN/ha, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates (n = 4). Letters after measurement values indicate statistical (p < 0,05) significance between treatments. Bonferroni correction was used for post-hoc pairwise comparison.

		SPAD 1[1]	SPAD 2	C%	N%	C/N	LAI[1.1]
A		34.073	32.4	44.476	3.695	21.322	0.3
B		34.9	32.6	43.186	2.502	21.052	0.8
C		36.9	33.6	43.177	2.588	20.285	1.0
D		35.6	32.2	42.108	3.382	17.47	0.9
E		35.6	33.9	43.527	2.038	23.991	1.1
1		34.5	32.2	43.122	2.65	20.749	0.9
2		35.1	32.7	43.362	2.806	20.488	0.1
3		36.7**a**	33.9	43.4	3.067	21.234	1.0
	df	**p-values**					
BC	**4**	0.644	0.784	0.421	0.195	0.392	0.131
Fert	**2**	**0.037**	0.162	0.700	0.186	0.554	0.267
BC x Fert	**8**	0.750	0.357	0.448	0.308	0.607	0.889

[1]Data was normally distributed according to normality tests of residuals, but it contained one abnormally high SPAD value of 44,10 for treatment type D1 (plot 79).
[1.1]No measurement results from plot 15 (treatment type D2).

5.4. Barley biomass and yield components

Both fertilization treatments significantly (p < 0.05) increased biomass, seeds per plant, thousand seed weight and final yield in comparison to the control (Table 11). However, for biomass, seeds per plant, 1000 seed weight and final yield, mineral fertilization seemed to increase these more (p < 0.001, 0.002, < 0.001 and < 0.001, respectively) in comparison to the control than the organic fertilizer (p 0.004, 0.054, 0.001 and 0.002, respectively). No significant (p < 0.05) effects of fertilization were observed on plants per square meter, seeds per ear or on ears per plant (Table 11). For harvest index (HI), the significance of fertilization was p 0.054. Biochar did not have any significant effects on any measured yield components of barley, nor were any significant combined effects observed together with fertilization (Table 11).

Table 11. Barley biomass and yield components by treatment type means. Biochar (BC) application rates are A–E (A = 0 t/ha, B = 5 t/ha, C = 10 t/ha, D = 20 t/ha, E = 30 t/ha) and fertilizer (Fert) treatments 1–3 (1 = control, 2 = MBM 100 kgN/ha, 3 = mineral fertilizer 100 kgN/ha). The results are the means from four replicates (n = 4). Letters after measurement values indicate statistical (p < 0,05) significance between treatments. Bonferroni correction was used for post-hoc pairwise comparison.

Treatment		Biomass (g/m^2)	Plants/m^2	Seeds/ plant[1]	Seeds/ ear[1]	Ears/ plant	HI	Yield (t/ha)	1000 seed weight (g)
A		282.8	733.7	6.5	7.40	0.90	0.49	1.3	26.1
B		344.6	832.1	7.3	7.84	0.91	0.50	1.6	26.9
C		304.0	822.7	5.8	7.41	0.77	0.42	1.3	24.5
D		274.1	763.1	5.9	7.15	0.80	0.42	1.2	23.5
E		392.1	859.5	8.4	8.58	0.97	0.53	1.9	26.0
1		259.8**b**	758.8	5.9**b**	7.17	0.83	0.45	1.1**b**	23.8**b**
2		334.6**a**	813.3	6.9**a**	7.53	0.90	0.48	1.5	26.1
3		364.1	834.6	7.4	8.32	0.88	0.48	1.7**a**	26.3**a**
	df	**p-values**							
BC	**4**	0.512	0.441	0.611	0.759	0.166	0.263	0.421	0.255
Fert	**2**	**<0.001**	**0.002**	*0.081*	0.126	0.109	*0.054*	**<0.001**	**<0.001**
BC x Fert	**8**	0.667	0.679	0.923	0.425	0.138	0.507	0.611	0.633

[1]Data was not normally distributed even after 3 Box-cox transformations, so p-values from non-transformed data were used for these variables.

6 DISCUSSION

6.1. Content of soil moisture and nutrients

The sandy soil texture of the research field has low water retention capacity, and during the growing season 2018, soil moisture content was near or below wilting point for several weeks during important development stages of the crop. Biochar did not significantly affect the volumetric moisture content of the soil at any application rate or at any analyzed depth. As biochars mostly affect soil water retention capacity in soils with low SOM-contents, as adding biochar increases soil C-content. This most likely explains why the effects in this study were not significant, as the research field has relatively high C-content. In some cases, biochar has been found to temporarily decrease soil moisture, which could be one possible reason for why the 10 t/ha treatment plots had lower soil moisture on some measurement dates even in comparison to the control. In addition, the number and species of plants may also affect soil moisture contents especially in the topsoil via shading and transpiration, which could possibly have affected soil moisture at 0–15 cm during the most critical drought period.

This study was done eight years after the addition of biochar, so the effectiveness of the biochar was obviously decreased due to weathering or misplacement in the soil by soil management. However, similar results as obtained in 2018 have also been observed from the same research field in previous years. For example, Tammeorg et al. (2014b) also observed a similar significant difference in soil moisture between biochar treatments 10 t/ha and 30 t/ha as was observed in measurements in 2018, although more plant available water and higher water retention capacity were observed in the first years of the experiment. In addition, in 2014 (Lehti 2015) and 2016 (Hämäläinen 2018), 30 t/ha treatment plots had higher soil moisture than 10 t/ha plots, although the difference was non-significant.

Biochar had significant effects on pH, some, but not significant, effect on Ca, and significant effects on soil C/N-ratio. However, pairwise comparison between biochar treatments for these effects were all non-significant. Studies have shown that biochar can increase soil pH, but although the biochar added to Vadelmakallio eight years ago did have a pH of 8.1, have the observable effects on soil pH in previous years been non-significant (Hämäläinen 2018). In addition, the liming efficacy of the added biochar was low (< 1 % of $CaCO_3$; Table 3), and in this study, the lowest biochar treatment slightly decreased soil pH in comparison to the control. Also, the effect of the covariate in the analyses, soil C-content before the addition of biochar in 2011 was significant (p 0.032) for soil pH in 2018, which might have affected this result.

Soil C/N was significantly affected by biochar, and 30 t/ha treatment plots contained more C than plots with 10 t/ha. The difference was, however, not significant, as was not the difference between control and 10 t/ha. The non-significant difference between control and 10 t/ha may be due to misplacement or microbial or mechanical degradation of the added biochar, which can be more noticeable in plots with lower application rates.

Both fertilization treatments affected soil nutrient contents, conductivity and pH. The amount of N, P and K were equal in both fertilizer treatments, but plots with MBM contained more P than plots with mineral fertilization. Similar results have been obtained in previous years (Hämäläinen 2018) and might be due to the slower release of P into the soil from MBM, which can cause soil P content to slowly increase with continuous use (Ylivainio and Turtola 2009). MBM plots had more Ca than mineral fertilizer plots, which is most likely due to the fact that the fertilizer itself contains more Ca. Mineral fertilization increased soil K, S and Mn significantly more than MBM fertilization, which can be due to slower mineralization of nutrients from MBM in dry soil (Borowik and Wyszkowska 2016).

Significant interactions between mineral fertilization and biochar were observed for B and Cu. Biochar treatment 30 t/ha with mineral fertilization significantly increased soil B, but biochar treatment 10 t/ha combined with no fertilization significantly lowered soil B. Similar effects were observed with Cu. For B, lower soil moisture combined with coarse soil texture may have reduced its availability (Wear and Patterson 1961) on control plots. The lower amount of Cu in soil may be due to its tendency to adsorb to organic matter (Mengel et al. 2001), and in this case, the biochar particles.

6.2. Barley leaf area index, C/N-ratio, SPAD value, biomass and yield components

Many studies have been conducted on biochars' effects on crop yields, but fewer studies have focused on the effects on yield components. Although in certain studies biochar has been shown to have positive effects on cereal yield components, such as increased biomass and grain yield in durum wheat (*Triticum durum* Desf.) (Vaccari et al. 2011), number of tillers and yield in rice (Bakar et al. 2015), yield and cob weight in maize (Sara & Shah 2018), increased panicle size and number of panicles in rice (Huang et al. 2019), other studies have observed no effects on these (Tammeorg et al. 2014b, Nelissen et al. 2015, Sänger 2016). This corresponds with the results obtained in this study, where no effects on yield components, LAI, C/N-ratio or SPAD value were observed.

Higher average temperatures combined with less than average rainfall negatively affected crop yield on the research site in 2018. According to weather data (Figure 6) and soil moisture measurements, crop growth stages related to flowering occurred at the same time with the most critical drought in July, and low soil moisture during emergence combined with weeds may have slowed down the growth and development of the plants. Biochars' effects on crop yield components are typically indirect by, for example, increasing soil water retention capacity and decreasing water stress, but as no effects on soil moisture of any biochar application rates were observed in this study, the negative effects of drought on yield formation were apparent. During the growing season of 2018, drought and high temperatures affected yield components at all critical development stages: tillering and prior to flowering (Aspinall et al. 1964, Slafer 2002, Samarah 2005). The number of grains per ear and per plant were lower than average (Rajala and Peltonen-Sainio 2018), and grain weight was also lower than average (Rajala 2018), likely due to the fact that drought during and slightly after flowering decreases grain size (Aspinall et al. 1964).

Drought most likely also negatively affected the SPAD values, as visible wilting and yellowing of crop leaves were observed during measurements. For SPAD and crop C/N-ratio and contents, significant effects were only observed on the first SPAD measurement, where only mineral fertilization increased leaf chlorophyll contents in comparison to the control and MBM. This might be due to the fact that nutrients in mineral form are more easily available to plants than nutrients in organic fertilizers, as organic compounds need to be mineralized by microbes (Borowik and Wyszkowska 2016). Microbial activity is also slowed down in dry conditions, which can further hinder the availability of nutrients from organic fertilizers (Stark and Firestone 1995, Borowik and Wyszkowska 2016).

Biochar alone did not have any significant effects on barley yield components but both fertilization treatments did significantly increase biomass, number of seeds per plant, thousand seed weight and final yield in comparison to the control. However, as with SPAD values, mineral fertilization affected these yield components more than organic fertilization, as mineralization is slowed down in dry conditions (Stark and Firestone 1995, Borowik and Wyszkowska 2016). However, plants/m_2, ears per plant or seeds per ear were not affected by any fertilization treatments, which is likely due to the fact that these are more determined by other factors, such as sowing time and technique for the plant density (Kirby 1969) and plant density for grains per ear (AHDB 2018). Harvest index was positively affected by fertilization (p 0.054), but not significantly. Harvest index represents the relationship between economically valuable yield and the total yield, and it is therefore affected by other yield components (number of grains and total yield). Nutrient availability in soil generally increase crop yield (Oscarsson et al. 1998), but weather conditions and disturbance from weeds or insects during the growing season may impact the results of the study, as these affect the growing conditions and growth of the crop.

7 CONCLUSIONS

The aim of this study was to determine if softwood biochar can affect soil properties or yield components of barley alone or together with different fertilizers 8 years after the biochar's application. There were observable effects of the added biochar eight years after application. However, the effects observed were not significant at any application rate, which could indicate that even higher rates than 30 t/ha may be needed to achieve significant changes in soil moisture retention in boreal loamy soils. Based on this and previous years' studies on Vadelmakallio research field, the addition of up to 30 t/ha of softwood biochar does not have negative effects on soil nutrient contents, pH or electrical conductivity in the long-term, which indicates that biochar is safe to use as a soil amendment or as a way to sequester carbon into the soil. Furthermore, no negative effects of any biochar application rate were found on the yield components, LAI, leaf chlorophyll contents or on the contents of C, N, and C/N-ratio of barley in boreal conditions.

Both mineral and organic fertilization increased certain yield components of barley, but nutrients from organic MBM fertilization may be less readily available to crops in very dry conditions. In addition, continuous use of MBM may cause a build-up of soil P contents, which needs to be taken into consideration when adding fertilizers to crops. Biochar and fertilization may have combined effects on the availability of certain soil nutrients (Mg, B, Cu). However, similar results have not been obtained in previous years, which could indicate that these interactions between fertilization and biochar are present only in very dry conditions.

Although, based on this year's (2018) study together with previous years' studies in Vadelmakallio field, the use of biochar in boreal conditions does not have negative effects on soil or crop properties, more research is still needed to determine sufficient application rates for achieving significant positive effects on soil properties and on crop yield components. In addition, it would be beneficial to conduct more research on the effects and interactions of different biochars together with different organic fertilizers in boreal conditions, as the use of different organic fertilizers is steadily increasing and not much is known on how these may interact. Furthermore, research in the future could also be more concentrated on the effects of fertilization and biochars on yield components of different crops, as gaining more insight into the mechanisms that affect and make up the total yield are beneficial to developing sustainable agriculture.

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